

A1  
concl. Winston; U.S. Patent No. 5,468,715 filed June 2, 1993 to Joseph et al.; U.S. Patent Nos. 5,468,716 filed October 3, 1994, 5,496,568 filed June 26, 1995, 5,518,986 filed April 4, 1995, 5,518,987 filed October 3, 1995 and 5,583,089 filed May 9, 1995 to Winston.

Please amend the paragraph beginning on page 16, line 11 as follows:

**Composition of Formulation 1 (Dried Spent Brewer's Grain):** Spent brewer's grain obtained from a local brewery was spread out on trays and allowed to air dry overnight. The dried spent brewer's grain was then added to soil that contained 20% moisture (12 grams dried spent brewer's grain per 100 grams moist soil).

A2 [Please amend the paragraph beginning on page 16, line 18 as follows:]

**Trap Design:** Jar traps were constructed from 16 ounce polyethylene jars with plastic screw caps. Each jar was drilled with 36 evenly-spaced holes (3 mm diameter) to allow volatiles to diffuse out of the trap and to allow termites to enter. A cylindrical basket was constructed for each cup trap from plastic fencing to facilitate removing the trap from the soil. Baited traps were prepared by placing 300 grams of Formulation 1 in a jar trap. Unbaited traps were filled with 300 grams of soil (20% moisture). A disk of cardboard (8 cm diameter) was placed in the top of each trap (baited and unbaited), covered with a thin layer of soil, and the lid was then screwed onto the trap.

Please amend the paragraph beginning on page 17, line 14 as follows:

**Results:**

- A3
1. Traps baited with dried spent brewer's grain (Formulation 1) were discovered sooner by termites than unbaited traps (Graph 1A).
  2. Termites consumed more cardboard from baited traps than from unbaited traps (Graph 1B).
  3. Termites were found more often in the baited traps than the unbaited traps (data collected, but not shown here).

Please amend the paragraph beginning on page 20, line 3 as follows:

**Composition of Formulation 2 (Dried Ground Germinated Corn**

A4 **Seeds):** Corn seeds were soaked in soapy water overnight, rinsed well and germinated in a

A4  
concl.

covered plastic tub containing moist germination paper. After 3 days of germination, the germinating corn was ground to meal using a kitchen food processor, then spread out on trays and allowed to air dry overnight. Dried, ground, germinated corn seed (12 grams per 100 grams soil) was added to soil that contained 20% moisture.

Please amend the paragraph beginning on page 20, line 14 as follows:

A5

**Trap Design:** Jar traps were constructed from 16 ounce polyethylene jars with plastic screw caps. Each jar was drilled with 36 evenly-spaced holes (3 mm diameter) to allow volatiles to diffuse out of the trap and to allow termites to enter. A cylindrical basket was constructed for each cup trap from plastic fencing to facilitate removing the trap from the soil. Baited traps were prepared by placing 300 grams of Formulation 2 in a jar trap. Unbaited traps were filled with 300 grams soil (20% moisture). A disk of cardboard (8 cm diameter) was placed in the top of each trap (baited and unbaited), covered with a thin layer of soil, and the lid was then screwed onto the trap.

Please amend the paragraph beginning on page 21, line 9 as follows:

**Results:**

- A6
1. The discovery time was shorter for the baited traps than for the unbaited traps (Graph 2A).
  2. More cardboard was consumed by termites in the baited traps for weeks 1 through 7 (Graph 2B).
  3. Termites were found more often in the baited traps than the unbaited traps (data collected, but not shown here).

Please amend the paragraph beginning on page 23, line 3 as follows:

A7

**Composition of Formulation 3 (Whole Dry Malted Barley):** Whole dry malted barley was obtained from a local brewer's store. The whole dry malted barley was then added to soil that contained 20% moisture (12 grams whole dry malted barley per 100 grams moist soil).

Please amend the paragraph beginning on page 23, line 9 as follows:

A8

**Trap Design:** Jar traps were constructed from 16 ounce polyethylene jars with plastic screw

A8  
concl.

caps. Each jar was drilled with 36 evenly-spaced holes (3 mm diameter) to allow volatiles to diffuse out of the trap and to allow termites to enter. A cylindrical basket was constructed for each cup trap from plastic fencing to facilitate removing the trap from the soil. Baited traps were prepared by placing 300 grams of Formulation 3 in a jar trap. Unbaited traps were filled with 300 grams of soil (20% moisture). A disk of cardboard (8 cm diameter) was placed in the top of each trap (baited and unbaited), covered with a thin layer of soil, and the lid was then screwed onto the trap.

Please amend the paragraph beginning on page 24, line 6 as follows:

**Results:**

- A9
1. Traps baited with whole malted barley (Formulation 3) were not discovered sooner by termites than unbaited traps (Graph 3A). Within 3 weeks, 10 baited and 10 unbaited traps had been discovered by termites.
  2. Termites did not consume more cardboard from baited traps than from unbaited traps (Graph 3B).
  3. Termites were not found more often in the baited traps than the unbaited traps (data collected, but not shown here).

Please amend the paragraph beginning on page 26, line 3 as follows:

A10

**Composition of Formulation 4 (Coated Sucrose Pellets):** Sucrose pellets with a light wax coating were obtained from a local supplier (Sprinkle Decorations, Wilton Enterprises, Woodridge, IL). The sucrose pellets with a light wax coating were then added to soil that contained 20% moisture (12 grams per 100 grams moist soil).

Please amend the paragraph beginning on page 26, line 10 as follows:

A11

**Trap Design:** Jar traps were constructed from 16 ounce polyethylene jars with plastic screw caps. Each jar was drilled with 36 evenly-spaced holes (3 mm diameter) to allow volatiles to diffuse out of the trap and to allow termites to enter. A cylindrical basket was constructed for each cup trap from plastic fencing to facilitate removing the trap from the soil. Baited traps were prepared by placing 300 grams of Formulation 4 in a jar trap. Unbaited traps were filled with 300 grams of soil (20% moisture). A disk of cardboard (8 cm diameter) was placed in the top of

A11  
concl. each trap (baited and unbaited), covered with a thin layer of soil, and the lid was then screwed onto the trap.

Please amend the paragraph beginning on page 27, line 9 as follows:

**Results:**

- A12
1. Traps baited with coated sucrose pellets (Formulation 4) were not discovered sooner by termites than unbaited traps (Graph 4A). Within 3 weeks, 10 baited and 10 unbaited traps had been discovered by termites.
  2. Termites did not consume more cardboard from baited traps than from unbaited traps (Graph 4B).
  3. Termites were not found more often in the baited traps than the unbaited traps (data collected, but not shown here).

Please amend the paragraph beginning on page 29, line 3 as follows:

**Composition of Formulation 1 (also denoted "F-1", and Dried Spent Brewer's Grain):**

A13 Spent brewer's grain obtained from a local brewery was spread out on trays and allowed to air dry overnight. The dried spent brewer's grain was then added to soil that contained 20% moisture (12 grams dried spent brewer's grain per 100 grams moist soil).

Please amend the paragraph beginning on page 29, line 10 as follows:

A14 Trap Design: Jar traps were constructed from 16 ounce polyethylene jars with plastic screw caps. Each jar was drilled with 36 evenly-spaced holes (3 mm diameter) to allow volatiles to diffuse out of the trap and to allow termites to enter. A cylindrical basket was constructed for each cup trap from plastic fencing to facilitate removing the trap from the soil. Baited traps were prepared by placing 300 grams of Formulation 1 in a jar trap. Unbaited traps were filled with 300 grams of soil (20% moisture). A pre-weighed square of Ponderosa pine (4 x 4 x 0.5 cm) was soaked in water for 15 minutes and was placed in the top of each trap (baited and unbaited), covered with a thin layer of soil, and the lid was then screwed onto the trap.

Please amend the paragraph beginning on page 30, line 7 as follows:

**Results:**

A15

A15  
correct.

1. Traps baited with dried spent brewer's grain (Formulation 1) were discovered sooner by termites than unbaited traps (Graph 5C).

2. Termites consumed more wood from baited traps than from unbaited traps (Graph 5B).

3. Termites were found more often in the baited traps than the unbaited traps (Graph 5A).

Please amend the paragraph beginning on page 32, line 3 as follows:

**Composition of Formulation 2 (also denoted "F-2" and Dried Ground Germinated Corn**

A16

**Seeds):** Corn seeds were soaked in soapy water overnight, rinsed well and germinated in a covered plastic tub containing moist germination paper. After 3 days of germination, the germinating corn was ground to meal using a kitchen food processor, than spread out on trays and allowed to air dry overnight. Dried, ground, germinated corn seed (12 grams per 100 grams soil) was added to soil that contained 20% moisture.

Please amend the paragraph beginning on page 32, line 13 as follows:

A17

**Trap Design:** Jar traps were constructed from 16 ounce polyethylene jars with plastic screw caps. Each jar was drilled with 36 evenly-spaced holes (3 mm diameter) to allow volatiles to diffuse out of the trap and to allow termites to enter. A cylindrical basket was constructed for each cup trap from plastic fencing to facilitate removing the trap from the soil. Baited traps were prepared by placing 300 grams of Formulation 2 in a jar trap. Unbaited traps were filled with 300 grams soil (20% moisture). A pre-weighed square of Ponderosa pine (4 x 4 x 0.5 cm) was soaked in water for 15 minutes and was placed in the top of each trap (baited and unbaited), covered with a thin layer of soil, and the lid was then screwed onto the trap.

Please amend the paragraph beginning on page 33, line 10 as follows:

**Results:**

- A18
1. The discovery time was shorter for the baited traps than for the unbaited traps (Graph 6C).
  2. More wood was consumed by termites in the unbaited traps than from the baited traps for weeks 1 and 2, but more was consumed from the baited traps in weeks 3 and 4 (Graph 6B).
  3. Termites were found more often in the baited traps than the unbaited traps (Graph 6A).

Please amend the paragraph beginning on page 35, line 3 as follows:

A19  
**Composition of Formulation 5 (also denoted "F-5" and Fizzies Instant Sparkling Drink Tablets):** Effervescent tablets comprised of 50:50 citric acid:sodium bicarbonate were obtained from a local grocery store (Fizzies brand drink tablets, Premiere Innovations, Pacific Palisades, CA 90272). Two tablets (3 grams each) were added to soil (300 grams) that contained 20% moisture.

Please amend the paragraph beginning on page 35, line 11 as follows:

A20  
**Trap Design:** Jar traps were constructed from 16 ounce polyethylene jars with plastic screw caps. Each jar was drilled with 36 evenly-spaced holes (3 mm diameter) to allow volatiles to diffuse out of the trap and to allow termites to enter. A cylindrical basket was constructed for each cup trap from plastic fencing to facilitate removing the trap from the soil. Baited traps were prepared by placing 300 grams of Formulation 5 in a jar trap. Control traps were filled only with 300 grams soil (20% moisture). A square of Ponderosa pine (4 cm by 4 cm by 0.5 cm width) that had been pre-weighed was moistened by soaking it in water for 15 minutes, then placed in the top of each trap (baited and unbaited) just below the surface of the soil.

Please amend the paragraph beginning on page 36, line 8 as follows:

**Results:**

- A21
1. The discovery time was shorter for the baited traps than for the unbaited traps (Graph 7C).
  2. More wood was consumed by termites in the baited traps than from the unbaited traps (Graph 7B).
  3. Termites were found more often in the baited traps than the unbaited traps (Graph 7A).

Please amend the paragraph beginning on page 39, line 1 as follows:

A22  
**Preparation of Formulations:** A CO<sub>2</sub>-generating formulation was added to soil that contained 20% moisture. The amount of each formulation to be mixed with 100 grams soil is listed below. For each experiment, one cup was filled with 25 grams moist soil (20% water). The other cup was filled with formulation/soil mixture (25 grams total). A circle of corrugated cardboard (3 cm diameter) was moistened with water, blotted lightly and placed on top of soil. The lid was put on

A22  
conc).

and the cups were inverted.

Please amend the paragraph beginning on page 39, line 11 as follows:

A23

**Analysis of CO<sub>2</sub>:** A capillary tube (5.5 cm long, 0.5 mm diameter) was inserted into the hole in the top of the inverted plastic cup. CO<sub>2</sub> was measured by taking a sample of the atmosphere within the soil using a 10 microliter syringe. The CO<sub>2</sub> concentration was determined using gas chromatography-mass spectrometry with selected ion monitoring (GC-MS-SIM) at m/e 44. The cup was used for a behavioral bioassay after the CO<sub>2</sub> concentration was determined to be adequate. Some formulations required 24-36 hours to generate enough CO<sub>2</sub>.

Please amend the paragraph beginning on page 39, line 23 as follows:

**Formulation 1: Dried Spent Grain (0.5 grams per 25 grams soil):** Significantly more termites were recovered from the treated cups than the controls for both species of termites (treatment 1 of graphs 8A and B). The average CO<sub>2</sub> concentration at the start of the bioassay was 6.48 mmol per mol (treatment 1 of graphs 8C).

A24 [ Please amend the paragraph beginning on page 39, line 28 as follows: ]

**Formulation 2: Dried Ground Germinated Corn Seeds (0.5 grams per 25 grams soil):** Significantly more termites were recovered from the treated cups than the controls for *Reticulitermes tibialis* (treatment 2 of Graph 8A). Slightly more termites were recovered from the treated cups than the controls in tests with *Reticulitermes virginicus* (treatment 3 of Graph 3B). The average CO<sub>2</sub> concentration at the start of the bioassay was 5.55 mmol per mol (treatment 2 of Graph 8C).

Please amend the paragraph beginning on page 40, line 1 as follows:

A25

**Formulation 3: Whole, malted barley (0.5 grams per 25 grams soil):** Significantly more termites were recovered from the treated cups than the controls for *Reticulitermes tibialis* (Graph 8). Slightly more termites were recovered from the treated cups than the controls in tests with *Reticulitermes virginicus*. The average CO<sub>2</sub> concentration at the start of the bioassay was 3.7 mmol per mol (graph 8).

Please amend the paragraph beginning on page 40, line 9 as follows:

**Formulation 4:** Sucrose pellets with a light wax coating (0.5 grams per 25 grams soil): Significantly more termites were recovered from the treated cups than the controls for *Reticulitermes tibialis* (treatment 4 of Graph 8A). The average CO<sub>2</sub> concentration at the start of the bioassay was 5.22 mmol per mol (treatment 4 of Graph 8C).

[Please amend the paragraph beginning on page 40, line 15 as follows:]

**Formulation 5:** Effervescent tablets (Fizzies brand drink tablets, 0.25 grams per 25 grams soil): There was no significant difference in the number of termites recovered from the treatment and the control for *Reticulitermes tibialis* (treatment 5 of Graph 8C). The average CO<sub>2</sub> concentration at the start of the bioassay was 38.19 mmol per mol (treatment 5 of Graph 8C).

[Please amend the paragraph beginning on page 40, line 21 as follows:]

**Formulation 6:** Yeast Granules (made from corn flour, corn syrup, NYPD nutrient broth and baker's yeast, 0.5 grams granules per 25 grams soil): Significantly more termites were recovered from the treated cups than the controls for *Reticulitermes tibialis* (treatment 6 of Graph 8A). There was no significant difference in the number of termites recovered from the treatment and the control for *Reticulitermes virginicus* (treatment 6 of Graph 8B). The average CO<sub>2</sub> concentration at the start of the bioassay was 5.60 mmol per mol (treatment 6 of Graph 8C).

[Please amend the paragraph beginning on page 40, line 31 as follows:]

**Formulation 7:** Dry Baker's Yeast (0.25 grams granules per 25 grams soil): Significantly more termites were recovered from the treated cups than the controls for *Reticulitermes tibialis* (treatment 7 of Graph 8A). The average CO<sub>2</sub> concentration at the start of the bioassay was 5.93 mmol per mol (treatment 7 of Graph 8C).

[Please amend the paragraph beginning on page 41, line 1 as follows:]

**Formulation 8:** Potassium Bicarbonate, Fine Granules (0.25 grams granules per 25 grams soil): Significantly more termites were recovered from the treated cups than the controls for *Reticulitermes tibialis* (treatment 8 of Graph 8A). The average CO<sub>2</sub> concentration at the start of the bioassay was 16.71 mmol per mol (treatment of Graph 8C).



[Please amend the paragraph beginning on page 41, line 7 as follows:]

**Formulation 9:** Clean Cracked Corn (sold as livestock feed) (0.5 grams granules per 25 grams soil): Significantly more termites were recovered from the treated cups than the controls for *Reticulitermes tibialis* (treatment 9 of Graph 8A). The average CO<sub>2</sub> concentration at the start of the bioassay was 4.21 mmol per mol (treatment 9 of Graph 8C).

[Please amend the paragraph beginning on page 41, line 13 as follows:]

*A26*  
*cont.*  
**Formulation 10:** Ground Dry Corn Seed (0.5 grams granules per 25 grams soil): Significantly more termites were recovered from the treated cups than the controls for *Reticulitermes tibialis* (treatment 10 of Graph 8A). The average CO<sub>2</sub> concentration at the start of the bioassay was 4.48 mmol per mol (treatment 10 of Graph 8C).

[Please amend the paragraph beginning on page 41, line 19 as follows:]

**Formulation 11:** Ground Malted Barley (0.5 grams granules per 25 grams soil): There was no significant difference in the number of termites recovered from the treatment and the control for *Reticulitermes tibialis* (treatment 11 of Graph 8A). The average CO<sub>2</sub> concentration at the start of the bioassay was 8.31 mmol per mol (treatment 11 of Graph 8C).

[Please amend the paragraph beginning on page 41, line 25 as follows:]

**Formulation 12:** Baking Powder/Corn Syrup Granules (0.5 grams granules per 25 grams soil): These granules were made from double-acting baking powder and corn syrup. Significantly more termites were recovered from the treated cups than the controls for *Reticulitermes tibialis* (treatment 12 of Graph 8A). The average CO<sub>2</sub> concentration at the start of the bioassay was 18.86 mmol per mol (treatment 12 of Graph 8C).

[Please amend the paragraph beginning on page 41, line 33 as follows:]

**Conclusions:**

1. In laboratory behavioral bioassays, *Reticulitermes tibialis* exhibited attraction to formulations 1, 2, 3, 4, 6, 7, 8, 9, 10 and 12 (treatments 1, 2, 3, 4, 6, 7, 8, 9, 10 and 12 of Graph 8A). In this particular context, *Reticulitermes tibialis* were not attracted to formulation 5 or 11.

2. In laboratory bioassays, *Reticulitermes virginicus* exhibited attraction to formulations 1, and 2 (treatments 1 and 2 of Graph 8B). In this particular context, *Reticulitermes virginicus* were not attracted to formulation 3 or 4.

3. All the formulations contained elevated CO<sub>2</sub> by comparison with controls (i.e., control treatment of Graph 8C).

[Please amend the paragraph beginning on page 45, line 4 as follows:]

**Composition of Formulation 1:** Dried spent brewer's grain was obtained from a local brewery, and was spread out and allowed to air dry overnight. Dried spent grain (12 grams per 100 grams soil) was added to soil that contained 20% moisture.

[Please amend the paragraph beginning on page 45, line 10 as follows:]

**Trap Design:** Dow Sentricon Termite Bait Stations were used for field experiments. A perforated plastic sleeve of our own design was inserted into each Dow Sentricon Termite Bait Stations to allow CO<sub>2</sub> generating formulations to be used in them. The insert consisted of a tube (21 cm long, 3.5 cm diameter) constructed of clear acetate film. Holes were punched 3 cm apart in the tube (0.5 cm) to allow CO<sub>2</sub> to diffuse out and to allow termites to enter the trap. Baited traps were prepared by placing a strip of Dow Sentricon wood (18 cm by 2.5 cm by 0.5 cm) inside a perforated plastic sleeve, then adding 150 grams of Formulation 1. This thinner strip of Dow wood was necessary in order to allow Formulation 1 to fill the plastic sleeve properly. The filled sleeve was then inserted into a Dow Sentricon Termite Bait Station. Control traps contained perforated plastic sleeves filled with a strip of Dow Sentricon Wood and 150 grams soil (20% moisture).

Please amend the paragraph beginning on page 46, line 15 as follows:

**Results:**

1. Termites were present in the baited traps for all 6 weeks of the experiment (Graph 9, the first bar for each week).
2. Termites were present in the soil-only control traps during week 1, 4, and 6 (Graph 9, the second bar for each week).
3. Termites were not present in any of the Dow control traps during the entire 6 weeks (Graph 9,

A27  
(conc.) no bar is present in the third position of each week).

4. Feeding on the wood strips was heavier in the baited traps and in the soil-only control traps than in the unmodified Dow Sentricon Bait Stations (data collected, but not shown).

Please amend the paragraph beginning on page 49, line 4 as follows:

A28  
**Composition of Formulation 2:** Corn seeds were soaked in soapy water overnight, rinsed well and germinated in a covered plastic tub containing moist germination paper. After 3 days of germination, the germinating corn was ground to meal using a kitchen food processor, then spread out on trays and allowed to air dry overnight. Dried ground germinated corn seed (12 grams per 100 grams soil) was added to soil that contained 20% moisture.

Please amend the paragraph beginning on page 49, line 13 as follows:

A29  
Trap Design: Dow Sentricon Termite Bait Stations were used for field experiments. A perforated plastic sleeve of our own design was inserted into each Dow Sentricon Termite Bait Stations to allow CO<sub>2</sub> generating formulations to be used in them. The sleeve consisted of a tube (21 cm long, 3.5 cm diameter) constructed of clear acetate film. Holes were punched 3 cm apart in the tube (0.5 cm) to allow CO<sub>2</sub> to diffuse out and to allow termites to enter the trap. Baited 70 traps were prepared by placing a strip of Dow Sentricon Wood (18 cm by 2.5 cm by 0.5 cm) inside a perforated plastic sleeve, then adding 150 grams of Formulation 2. This thinner strip of Dow Sentricon Wood was necessary in order to allow Formulation 2 to fill the plastic sleeve properly. The filled sleeve was then inserted into a Dow Sentricon Termite Bait Station. Control traps contained perforated plastic sleeves filled with a strip of Dow Sentricon Wood and 150 grams soil (20% moisture).

Please amend the paragraph beginning on page 52, line 4 as follows:

A30  
**Composition of Formulation 4:** Sucrose pellets with a light wax coating were obtained from a local supplier (Sprinkle Decorations, Wilton Enterprises, Woodridge, IL). The sucrose pellets with a light wax coating were then added to soil that contained 20% moisture (12 grams per 100 grams moist soil).

Please amend the paragraph beginning on page 52, line 11 as follows:

A31  
**Trap Design:** Dow Sentricon Termite Bait Stations were used for field experiments. A perforated plastic sleeve of our own design was inserted into each Dow Sentricon Termite Bait Stations to allow CO<sub>2</sub> generating formulations to be used in them. The sleeve consisted of a tube (21 cm long, 3.5 cm diameter) constructed of clear acetate film. Holes were punched 3 cm apart in the tube (0.5 cm) to allow CO<sub>2</sub> to diffuse out and to allow termites to enter the trap. Baited traps were prepared by placing a strip of Dow Sentricon Wood (18 cm by 2.5 cm by 0.5 cm) inside a perforated plastic sleeve, then adding 150 grams of Formulation 4. This thinner strip of Dow Sentricon Wood was necessary in order to allow Formulation 4 to fill the plastic sleeve properly. The filled sleeve was then inserted into a Dow Sentricon Termite Bait Station. Control traps contained perforated plastic sleeves filled with a strip of Dow Sentricon Wood and 150 grams soil (20% moisture).

Please amend the paragraph beginning on page 57, line 4 as follows:

(n.e.) 2001. A35

**Results:**

- A32
1. *Reticulitermes tibialis* was attracted to 5, 10, and 20 mmol per mol CO<sub>2</sub>. (Graph 12B). *R. tibialis* demonstrated the best attraction to 5 mmol per mol CO<sub>2</sub> (Graph 12B).
  2. *Reticulitermes flavipes* was attracted to 2, 5 and 10 mmol per mol CO<sub>2</sub>. *R. flavipes* was most attracted to 10 mmol per mol (Graph 12A).
  3. *Reticulitermes virginicus* was attracted to 5, 10, 20 and 50 mmol per mol CO<sub>2</sub>. *R. virginicus* demonstrated best attraction to 10 mmol per mol CO<sub>2</sub> ().

Please amend the paragraph beginning on page 55, line 20 as follows:

A32  
 Mixtures of CO<sub>2</sub> and ambient air were tested to determine the termite response to a range of CO<sub>2</sub> concentrations. A 35-ml syringe was rinsed with distilled water and partially filled (5 ml) with ambient air. Different amounts of 100% CO<sub>2</sub> were obtained with a smaller glass syringe from a tank and injected into the 35-ml syringe. Ambient air was then drawn into the 35-ml syringe to fill it and mix the gases by turbulence as the syringe was loaded. A 2nd 35-ml polyethylene syringe was filled with ambient air for a control. Measurements with GC-MS-SIM confirmed that the CO<sub>2</sub> concentrations reached equilibrium after 15 minutes. The CO<sub>2</sub> concentration of the syringes was determined by using GC-MS-SIM analysis (see below) before each bioassay. Bioassays were conducted with both *Reticulitermes tibialis* and *Reticulitermes flavipes* for 1, 2,

A32  
(cnc).

5, 10, 20, 50 and 500 mmol per mol concentrations of CO<sub>2</sub> and with *Reticulitermes virginicus* for 5, 10, 20, and 50 mmol per mol.

Please amend the paragraph beginning on page 56, line 4 as follows:

A35

**Procedure:** For bioassays, termite workers were collected from plastic tubs by using a camel's hair brush and were placed into a holding container constructed from a 3 cm length of Teflon tubing (8 mm inside diameter). The container was plugged at one end with a NMR cap with two holes (1 mm) drilled in the bottom. A second NMR cap with a 4 mm hole was inserted backwards into the other end of the Teflon tube. The end of the NMR cap was sealed with a small square of cellophane held in place with a plastic tube (a piece of plastic soda straw) that fit snugly over the open end. Termites (5 workers) were placed in the container and the top was sealed. The container was placed horizontally and left undisturbed for 20 min. The T-tube apparatus was assembled and clamped horizontally on top of a block of foam rubber (12 by 12 cm) with a wire bent into a U-shape. The syringe pump was turned on, and after 3 min of pumping, the cellophane seal was removed from the holding container and the entrance to the holding container was gently connected to the central arm of the T-tube, allowing termites to crawl out and enter the apparatus. Bioassays were conducted for 15 minutes, after which the number of termites in each pitfall was recorded.

Please amend the paragraph beginning on page 57, line 4 as follows:

**Results:**

1. *Reticulitermes tibialis* was attracted to 2, 5, and 10 mmol per mol CO<sub>2</sub>. *R. tibialis* demonstrated the best attraction to 5 mmol per mol CO<sub>2</sub> (example 12, page 3).
2. *Reticulitermes flavipes* was attracted to 5, 10 and 20 mmol per mol CO<sub>2</sub>. *R. flavipes* was most attracted to 10 mmol per mol (example 12, page 3).
3. *Reticulitermes virginicus* was attracted to 5, 10, 20 and 50 mmol per mol CO<sub>2</sub>. *R. virginicus* demonstrated best attraction to 5 mmol per mol CO<sub>2</sub> (example 12, page 4).

Please amend the paragraph beginning on page 68, line 2 as follows:

A35

We showed in a laboratory behavioral bioassay that the termite *Reticulitermes tibialis* is attracted to CO<sub>2</sub>, in which we used a test concentration of 5 mmol/mol, or 0.5% CO<sub>2</sub> in air. Our

A35  
concl.

behavioral bioassay design involved a glass T-tube (5 mmID), modified with a laboratory torch so that the ends of the two choice arms projected down at 45 degrees angles from horizontal, to provide pitfalls after the termites made a choice. A syringe pump was used with two 35 ml polyethylene syringes, one filled with ambient air and the other filled with 5 mmol/mol CO<sub>2</sub> in air. Teflon tubing conveyed the odors to the two arms of the T-tube, at 1.0 ml/minute into each arm. We used a bubble meter to verify that the outflow from the center arm was 2.00 ml/minute, to assure that there were no leaks. We allowed the syringe pump to run for 3 minutes immediately before the bioassay began, to allow the flow rates and gas concentrations inside the T-tube to come to equilibrium. The body of the T-tube was mounted horizontally on a foam rubber block. A group of 5 termites was placed inside a small Teflon holding tube for 15 minutes. To allow them to acclimate to the bioassay environment (NMR caps with small holes in them to allow gas flow were used to plug the ends of the holding tube). The acclimation period and the bioassay itself were done under reduced lighting. After the 15 minutes acclimation period, an NMR cap was removed from one end of the holding tube, and the tube was connected to the center arm of the T-tube. Typical responses of the termites in the T-tube were consistent with our conclusion that the term "attraction is the correct interpretation of their behavior. When a termite came to the choice point, it moved its antennae to one side and then the other, finally making a choice toward the CO<sub>2</sub> side. The side on which CO<sub>2</sub> was presented is randomized from replication to replication, to control for possible side-to-side bias in the bioassay. After making a choice, the termite moved along the arm about 2 cm to where the dropped off at 45 degrees, and slid down the chute into the pitfall. The number of termites that was attracted to the CO<sub>2</sub> side of the bioassay was significantly greater than the number that moved to the control side.

---

Please amend the paragraph beginning on page 71, line 9 as follow:

A36

**Bioassay Apparatus.** The choice-test bioassay apparatus was constructed from a glass T-tube (5 mm inside diameter, 5 mm stem, with each branch 4.5 cm long). Each branch of the 'T' was bent downward (2 cm from junction of the T) at a 45 degree angle to form a 2.5 cm pitfall trap. A 5 mm NMR cap (cat. no. 100-0050, Drummond Scientific, Broomall, PA) with a 1 mm pinhole in it was firmly pushed over the end of each bent branch. A 25 cm length of Teflon tubing (0.8 mm ID) was inserted (3 mm) into the pinhole of each NMR cap and the other end of

A36  
concl. the tubing was connected to a 35 ml polyethylene syringe (cat no. 106-0490, Sherwood Medical, St. Louis, MO). The two 35-ml polyethylene syringes used for each bioassay were connected to a syringe pump (Sage Model 355, Fisher Scientific, Pittsburgh, PA) which was adjusted to provide an airflow of 1.0 ml/minute into each choice arm of the bioassay apparatus.

Please amend the paragraph beginning on page 74, line 6 through page 76, line 2 as follows

(Note the start of a new paragraph beginning at the phrase **WESTERN CORN**

**ROOTWORM**):

A37 A behavioral bioassay was developed to test responses of newly hatched (neonate) larvae of western corn root worm *Diabrotica virgifera virgifera* LeConte to volatile compounds from corn plants, a major host for this insect. A glass Y-tube filled with glass beads was used to allow choice tests in a vertical direction and to reproduce the thigmotactic cues available to larvae in their natural soil environment. A syringe pump was used to provide slow, consistent delivery of candidate compounds to the 2 sides of the apparatus. Significantly more larvae were attracted to the side containing a germinating corn seed than to the side containing ambient air. In addition, significantly more larvae were attracted to the side containing cut corn roots than to the side containing an ambient air control. Carbon dioxide (CO<sub>2</sub>) from corn roots previously has been implicated as an attractant for the larvae, and dose--response curves for larval attraction to CO<sub>2</sub> were obtained using different sources (different dilutions of carbonated water, the headspace over a carbonated water dilution, and different concentrations of CO<sub>2</sub> in air). The CO<sub>2</sub> concentrations for all sources were measured by mass spectrometry with selected ion monitoring at m/e 44. Neonate larvae were significantly attracted to concentrations of CO<sub>2</sub> as low as  $1.125 \pm 0.04$  mmol/mol (concentration of CO<sub>2</sub> in ambient air on the control side was  $0.99 \pm 0.02$  mmol/mol). Larvae were optimally attracted to 2.51--4.20 mmol/mol CO<sub>2</sub>, but they were attracted to concentrations as high as 100 mmol/mol. Larvae were not attracted to 300 or 900 mmol/mol CO<sub>2</sub>, and they exhibited toxic symptoms at these high concentrations. The concentration of CO<sub>2</sub> in soil near growing corn roots was  $4.36 \pm 0.31$  mmol/mol, which was consistent with the behavioral optimum for the larvae. The concentration of CO<sub>2</sub> in soil that contained no corn was  $1.38 \pm 0.03$  mmol/mol and the concentration in ambient air was  $0.94 \pm 0.01$  mmol/mol.

WESTERN CORN ROOT WORM, *Diabrotica virgifera virgifera* LeConte, is a major

A39  
concl.

pest of corn, *Zea mays* L., in the United States (Krysan and Miller 1986). The larvae can survive only on corn and a few other species of Poaceae (Branson and Ortman 1967, 1970), and they have been reported to move as far as 1 meter through the soil to find roots of a suitable host (Short and Luedtke 1970). Overwintering eggs hatch in the spring, and larvae must crawl through the soil to locate the roots on which they feed. One of the most important cues used by these larvae to locate corn roots is carbon dioxide (CO<sub>2</sub>), which is given off by corn roots in the soil (Harris and Van Bavel 1957, Massimino et al. 1980, Desjardins 1985, Labouriau and Jose 1987). Strnad et al. (1986) first reported that western corn root worm larvae are highly attracted to CO<sub>2</sub>, and subsequent investigators have confirmed this attraction (Hibbard and Bjostad 1988, MacDonald and Ellis 1990, Strnad and Dunn 1990, Jewett and Bjostad 1996). In laboratory bioassays, Hibbard and Bjostad (1988) showed that a cryogenic collection of volatile compounds from germinating corn seeds was attractive to 2nd instars of western corn root worm, and that CO<sub>2</sub> was present in the cryogenic collections. Jewett and Bjostad (1996) showed that dichloromethane is attractive to *Diabrotica* larvae, apparently because the structure of dichloromethane mimics CO<sub>2</sub> in its interaction with larval chemoreceptors.

---

Please amend the paragraph beginning on page 77, line 21 as follows:

A38

**Corn.** Untreated, dried corn seeds (*Zea mays* L., cv 3055 provided courtesy of Gary D. Lawrance, Pioneer Hi-Bred International, Inc., Johnston, IA) were washed with liquid soap, soaked for 24 hours in soapy water (1 drop of Ivory dishwashing liquid, Procter & Gamble, Cincinnati, OH, per liter of water), and rinsed thoroughly with water. For use in bioassays, the washed seeds were germinated 3 d on germination paper (Steel Blue, Anchor Paper, St. Paul, MN) in a closed polyethylene tub (30 by 15 cm). The plants typically reached a shoot length of 1 cm and a root length of 6 cm.

---

Please amend the paragraph beginning on page 77, line 32 as follows:

A39

**Bioassay Apparatus.** The choice-test bioassay apparatus (Graph 18-1A) was constructed from a glass Y-tube filled with glass beads to simulate the thigmotactic cues of the soil environment that are ordinarily encountered by western corn root worm larvae. The glass Y-tube was fabricated by a local glassblower (9.5 mm inside diameter, 60° angles, with each branch 3 cm long), and clamped to a ring stand with 2 branches of the "Y" facing down. A glass connection tube (4 cm



A39  
conc.)

long, 0.5 cm diameter) with a piece of vinyl screen (2.5-mm mesh) held over 1 end by a 0.5-cm section of Teflon tubing (6 mm inside diameter) was inserted snugly into the end of each of the arms of the Y-tube to support the glass beads. Glass beads (3 mm, cat. no. 11-312A, Fisher Scientific, Pittsburgh, PA) were poured into the top of the Y-tube, filling the entire apparatus to within 0.5 cm of the top (250 beads). A 5-mm NMR tube cap (cat. no. 100-0050, Drummond Scientific, Broomall, PA) was fitted into the other end of each glass connection tube, with a hole to allow snug insertion of a 20-cm piece of slender Teflon tubing (0.8 mm inside diameter) for introduction of volatile chemical cues into each arm of the bioassay apparatus. Two techniques were used to introduce candidate chemical cues into the 2 arms of the apparatus: 1 used shell vials as chemical sources, and the other used syringes as chemical sources.

Please amend the paragraph beginning on page 78, line 25 as follows:

A40

**Shell Vial Sources.** In this 1st approach (Graph 18-1A), two 35-ml polyethylene syringes (cat. no. 106-0490, Sherwood Medical, St. Louis, MO) were filled with ambient air, and the air was pumped through shell vials containing candidate chemical cues. Glass shell vials (4 ml) with polyethylene caps were used (cat. no. B7785-1, Baxter Healthcare, McGaw Park, IL). A 35-ml syringe was snugly connected with slender Teflon tubing (20 cm) to a hole in the cap of the shell vial. A 2nd piece of slender Teflon tubing was used to connect the shell vial to 1 arm of the bioassay apparatus. The 2 syringes used for each bioassay were connected to a syringe pump (Sage Model 355, Fisher Scientific, Pittsburgh, PA) that provided an airflow through each shell vial containing a candidate chemical treatment, and subsequently into a choice arm of the bioassay apparatus. For the shell vial sources of candidate chemical compounds, the shell vial containing either a carbonated water dilution or a corn seed or cut corn roots was left open for 5 min to allow the gas concentrations to reach equilibrium. The vial was capped, and the syringe pump was started, providing an airflow of 1.0 ml/min from each syringe.

Please amend the paragraph beginning on page 79, line 14 as follows:

A41

**Syringe Sources.** In this 2nd approach (Graph 18-2A), 35-ml polyethylene syringes were filled directly with candidate chemical cues (such as the headspace from a container of germinating corn, a sample of CO<sub>2</sub> mixed with air, or the headspace from a bottle of carbonated water). Each of the 2 syringes was connected with slender Teflon tubing to 1 arm of the bioassay apparatus.

**A41**  
The 2 syringes used for each bioassay were connected to a syringe pump that was adjusted to provide an airflow of 1 ml/min from each syringe.

Please amend the paragraph beginning on page 79, line 24 as follows:

**A42**  
**Bioassay Procedure.** For bioassays, 20 newly hatched 1st instars (0--12 hours old) were collected from tubs containing eggs in soil (by using a camel's hair brush) and placed in a covered 5-mm NMR cap with 2 holes (1 mm diameter) drilled in the bottom (graphs 18-1A and 18-2A). These holes were temporarily plugged with a piece of wire bent into a U-shape. The open end of the NMR cap was sealed with a small square of cellophane held in place with a plastic tube (a piece of soda straw) that fit snugly over the open end. The Y-tube apparatus was assembled and filled with glass beads and the appropriate treatment and control sources (shell vials or syringes) were connected to the arms of the Y-tube. The syringe pump was set to provide a flow of 1 ml/min and turned on. A flow meter was used to verify that the flow exiting the top of the Y-tube was 2 ml/min, confirming the flow of volatiles through the apparatus and verifying that there were no leaks in the connections. If the flow was inadequate, all connections were inspected and secured, and the flow was rechecked. After 3 min of pumping, the wire piece blocking the 2 holes in the NMR cap was removed and the cap was placed in the top of the Y-tube, allowing larvae to crawl out through the 2 holes and down into the glass beads. Bioassays were conducted for 30 min. The entire Y-apparatus was disassembled, and the positions of the larvae were recorded. Larvae were not reused in subsequent tests. Before each test, all glass parts of the apparatus were washed with soap and water, rinsed with water, and heated at 80°C in an oven for 30 min.

Please amend the paragraph beginning on page 80, line 30 as follows:

**A43**  
**Germinating Corn Seed Versus Air.** Using the shell vial source technique, germinating corn seeds were tested to determine whether larvae could detect volatile compounds produced by the growing seeds and follow them through a glass bead medium to the source. Individual washed corn seeds were placed in glass shell vials (4 ml) with a moistened piece of filter paper inside. The vials were placed on moistened germination paper inside a covered plastic container (30 by 15 cm) and germinated for 3 days. A vial containing a single 3-day-old germinating seed was removed from the covered plastic container just before testing and connected to the bioassay

A43  
conc).

apparatus. An empty shell vial was connected to the other side as a control. The CO<sub>2</sub> concentrations of the germinating corn seeds and the control were determined by using GC-MS-SIM.

---

Please amend the paragraph beginning on page 81, line 12 as follows:

A44

**Cut Corn Roots Versus Air.** In a companion experiment, cut corn roots were tested to determine whether larvae were attracted to volatile compounds produced by the roots alone. Corn roots (14.5 cm, 3 day old) were cut into 2--3 cm lengths and placed into 1 shell vial. The other shell vial (control side) contained ambient air. The CO<sub>2</sub> concentrations of the cut corn roots and the control were determined by using GC-MS-SIM.

---

Please amend the paragraph beginning on page 81, line 20 as follows:

A45

**Corn Headspace Bioassay.** Using the syringe source technique, the headspace over germinating corn seedlings was tested to determine the larval response to corn volatiles in the glass bead apparatus. Washed corn seeds were spread on moistened germination paper inside a covered plastic container (30 by 15 cm) and germinated for 3 days to allow volatile corn compounds to be produced. A 35-ml polyethylene syringe was filled with the headspace containing these volatile compounds by means of a 25 cm length of slender Teflon tubing inserted into a hole drilled into the cover. The control syringe was filled from an identical plastic container containing only moistened germination paper. The CO<sub>2</sub> concentrations of the syringes were determined by using GC-MS-SIM before each bioassay.

---

Please amend the paragraph beginning on page 82, line 3 as follows:

A46

**Consistency of CO<sub>2</sub> Delivery.** The consistency of the CO<sub>2</sub> concentration delivered into the bioassay apparatus was measured using GC-MS-SIM. For syringe sources, a 35-ml polyethylene syringe was partially filled with ambient air (5 ml) and 80 µl of CO<sub>2</sub> (obtained with a glass syringe from a tank containing pure CO<sub>2</sub>) was injected into the syringe. Ambient air was then drawn into the syringe to fill it, mixing the air and CO<sub>2</sub> thoroughly by turbulence at the same time. A syringe containing 800 µl of CO<sub>2</sub>, and another containing only ambient air, also were prepared. The syringes were allowed to equilibrate for 30 minutes before they were connected to the syringe pump (set at a flow of 1 ml/min). After 3 minutes of pumping, a 2-µl sample was

A46  
conc1.

taken from 5 cm inside a 20-cm length of Teflon tubing exiting from each syringe, by using a 10- $\mu$ l (Hamilton) syringe. To test consistency of CO<sub>2</sub> release from the syringes, samples were taken at 0, 10, 20, and 30 minutes (following the initial 3-minute pumping interval) and analyzed using GC-MS-SIM. For behavioral bioassays, samples were taken 5 minutes before the start of the bioassay from 5 cm inside the syringe.

---

Please amend the paragraph beginning on page 82, line 24 as follows:

---

A47

For shell vial sources, CO<sub>2</sub> concentrations were measured from the 0, 1, 3, 10, 30, and 100% dilutions of carbonated water. A dilution of carbonated water (1 ml) (see preparation below) was slowly dispensed into a shell vial (4 ml capacity) with a 1-ml Pasteur pipette. The vial was left open for 5 minutes to allow the CO<sub>2</sub> gas concentration to reach equilibrium. A 35-ml polyethylene syringe on the syringe pump was used to pump air through the shell vial at 1 ml/min. After 3 min of pumping, a 2- $\mu$ l sample of the headspace was taken from 5 cm inside a 20-cm length of Teflon tubing exiting from the shell vial, using a 10- $\mu$ l (Hamilton) syringe. To test consistency of CO<sub>2</sub> release from the shell vials, samples were taken at 0, 10, 20, and 30 minutes and analyzed using GC-MS-SIM.

[Please amend the paragraph beginning on page 83, line 6 as follows:]

**CO<sub>2</sub> Bioassay.** In a preliminary experiment, a 10-mmol/mol concentration of CO<sub>2</sub> was used to test larval attraction. A 35-ml polyethylene syringe was rinsed with distilled water to moisten the inside of the syringe, and partially filled (5 ml) with ambient air. The CO<sub>2</sub> (350  $\mu$ l) was obtained with a glass syringe from a tank containing pure (100%) CO<sub>2</sub> and injected into the 35-ml polyethylene syringe. Ambient air was then drawn into the syringe to fill it to a total volume of 35 ml, mixing the air and CO<sub>2</sub> thoroughly by turbulence. The gas mixture in the syringe was allowed to equilibrate for 15 minutes, and GC-MS-SIM was used to verify the CO<sub>2</sub> concentration before each bioassay. A 2nd 35-ml polyethylene syringe was filled with ambient air for a control, and the CO<sub>2</sub> concentration was measured using GC-MS-SIM.

[Please amend the paragraph beginning on page 83, line 21 as follows:]

**CO<sub>2</sub> (Dose—Response).** In subsequent experiments, mixtures of CO<sub>2</sub> and ambient air were tested to determine the larval response to a range of CO<sub>2</sub> concentrations. A 35-ml syringe was rinsed

A48  
c. ncl.  
with distilled water and partially filled (5 ml) with ambient air. Different amounts of 100% CO<sub>2</sub> were obtained with a smaller glass syringe from a tank and injected into the 35-ml syringe. Ambient air was then drawn into the 35-ml syringe to fill it and mix the gases by turbulence as the syringe was loaded. A 2nd 35-ml polyethylene syringe was filled with ambient air for a control. Measurements with GC-MS-SIM confirmed that the CO<sub>2</sub> concentrations reached equilibrium after 15 minutes. The CO<sub>2</sub> concentration of the syringes was determined by using GC-MS-SIM analysis before each bioassay.

---

Please amend the paragraph beginning on page 84, line 23 as follows:

---

A48  
**Diluted Carbonated Water (Dose--Response).** It has previously been shown that carbonated water can be used as a source of CO<sub>2</sub> to attract 2nd-instar western corn root worms (Jewett and Bjostad 1996). Dilutions of carbonated water (Canada Dry Club Soda, Cadbury Beverages, Stamford, CT) in distilled water were evaluated for attraction of western corn root worm larvae. With this approach, handling of carbonated water was conducted with slow pouring of large volumes of liquid, and all transfers into shell vials were made with large-diameter pipettes to minimize outgassing. Six concentrations of carbonated water (0, 1, 3, 10, 30, and 100%) were tested. A new, unopened bottle of carbonated water was used each day to prepare the dilutions. To prepare the 10 and 30% dilutions, the appropriate amount of distilled water was measured in a glass graduated cylinder and poured into a 300-ml glass bottle. The right amount of carbonated water was then measured in a graduated glass cylinder and poured slowly into the same bottle to minimize outgassing of CO<sub>2</sub>. The diluted mixture (150 ml total volume) was stirred gently with a glass rod. The 10 and 30% dilutions were used to prepare the 1 and 3% dilutions, respectively. For bioassays, each dilution of carbonated water (1 ml) was slowly dispensed into a shell vial (4 ml capacity) with a 1-ml Pasteur pipette. Distilled water (1 ml) was placed into a 2nd vial (control). The vials were left open for 5 minutes to allow the CO<sub>2</sub> gas concentration to reach equilibrium, then were connected to the bioassay apparatus. The CO<sub>2</sub> concentration in the headspace above the carbonated water dilutions in the shell vials was determined by using GC-MS-SIM.

---

Please amend the paragraph beginning on page 86, line 18 as follows:

**CO<sub>2</sub> Analysis of Corn Plants in Soil.** The bottom of a round, plastic tub (11 cm high, 17 cm diameter) was covered with 3 cm of soil, and 40 ml of water were added. Washed corn seeds (40--50) were distributed on top of the soil and the seeds were covered with an additional 3 cm of soil. The tubs were tightly covered. The lids were removed after 3 days, and the soil was kept slightly moist by adding water daily. Measurements of CO<sub>2</sub> were taken from the soil when the plants were 6--8 day old. A piece of metal wire (5.3 cm) was inserted into a glass tube (5 cm long, 1 mm inside diameter) so that the wire projected 3 mm from the end of the glass tube. The tube was inserted, wire first, 4 cm into the soil. The wire plug was removed from the glass tube, leaving a 3-mm gap in the soil just below the end of the glass tube. The needle of a 10- $\mu$ l Hamilton syringe was inserted into the glass tube so that it projected 1 mm into the gap, and a 5- $\mu$ l sample of soil headspace was removed. Samples were taken from different locations in the tub to minimize disturbance of the soil CO<sub>2</sub> concentrations. The CO<sub>2</sub> concentration of the soil headspace was determined by using GC-MS-SIM. Using the same method, samples were taken from control tubs containing soil alone.

Please amend the paragraph beginning on page 87, line 13 as follows:

**Germinating Corn Seed Versus Air Choice Test.** In experiments using shell vial sources, significantly more western corn root worm larvae ( $P < 0.05$ ) were attracted to the side containing the germinating corn seed than to the control side (Graph 18-1B). The CO<sub>2</sub> concentration of the headspace above the germinating corn seed was  $6.04 \pm 0.83$  (mean  $\pm$  SEM) mmol/mol, and the CO<sub>2</sub> concentration of the headspace on the control side was  $0.99 \pm 0.08$  mmol/mol (Graph 18-1D).

Please amend the paragraph beginning on page 87, line 22 as follows:

**Cut Corn Roots Versus Air Choice Test.** Significantly more western corn root worm larvae ( $P < 0.05$ ) were attracted to the side containing cut corn roots than to the control side (Graph 18-1C). The CO<sub>2</sub> concentration of the headspace above germinating corn roots was  $2.97 \pm 0.15$  mmol/mol, and the CO<sub>2</sub> concentration of the headspace on the control side was  $0.99 \pm 0.08$  mmol/mol (Graph 18-1E).

[Please amend the paragraph beginning on page 87, line 30 as follows:]

**Corn Headspace Bioassay.** In bioassays with syringe sources, significantly more western corn root worm larvae ( $P < 0.05$ ) were attracted to the side containing the headspace over germinating corn seeds than to the control side (Graph 18-2B). The  $\text{CO}_2$  concentration of the headspace above the germinating corn seeds was  $5.38 \pm 0.45$  mmol/mol, and the  $\text{CO}_2$  concentration of the headspace on the control side was  $1.14 \pm 0.13$  mmol/mol (Graph 18-2D).

Please amend the paragraph beginning on page 88, line 7 as follows:

**$\text{CO}_2$  Bioassay.** In a preliminary experiment to verify attraction of the larvae to syringe sources containing  $\text{CO}_2$ , significantly more western corn root worm larvae ( $P < 0.05$ ) were attracted to the side containing 10 mmol/mol  $\text{CO}_2$  ( $10.43 \pm 0.18$  mmol/mol) than to the control side (Graph 18-2C). The  $\text{CO}_2$  concentration of the control side was  $0.93 \pm 0.04$  mmol/mol (Graph 18-2E).

[Please amend the paragraph beginning on page 88, line 14 as follows:]

**Consistency of  $\text{CO}_2$  Delivery.** The release of  $\text{CO}_2$  from syringe sources was highly consistent over the course of a 30-min bioassay interval (Graph 18-3A). The release of  $\text{CO}_2$  from shell vial sources was consistent over the course of a 30 min bioassay interval for the lower doses tested (0, 1, 3, and 10%), but not for the higher doses (30 and 100%) (Graph 18-3B).

Please amend the paragraph beginning on page 88, line 31 as follows:

**$\text{CO}_2$  Selective Response.** Significantly more larvae were attracted (Graph 18-5) to the higher  $\text{CO}_2$  concentration for 1 versus 1.50 mmol/mol, for 2 versus 2.50 mmol/mol, for 5 versus 5.50 mmol/mol, and for 10 versus 10.50 mmol/mol, but no difference in attraction was observed for 20 versus 20.50 mmol/mol of  $\text{CO}_2$ . When smaller  $\text{CO}_2$  differences were tested (0.25 mmol/mol), fewer significant differences were observed. Larvae were more attracted to the higher  $\text{CO}_2$  concentration for 1 versus 1.25 mmol/mol, and for 2 versus 2.25 mmol/mol, but no difference in attraction was observed for 5 versus 5.25 mmol/mol, for 10 versus 10.25 mmol/mol, or for 20 versus 20.25 mmol/mol. At the smallest  $\text{CO}_2$  difference tested, significantly greater attraction was observed to 1.125 mmol/mol than to 1 mmol/mol (the actual  $\text{CO}_2$  concentration of the treatment side was  $1.18 \pm 0.05$  mmol/mol, and the actual control concentration was  $1.06 \pm 0.05$  mmol/mol), but no difference in attraction was observed in any of the tests at higher

AS3  
Control

concentrations. In control tests with equal amounts of CO<sub>2</sub> on both sides (1, 2, 5, 10, or 20 mmol/mol), no significant differences in attraction were observed.

Please amend the paragraph beginning on page 90, line 3 as follows:

AS4

**Shell Vial Control Bioassays.** There was no significant difference ( $P > 0.05$ ) between the numbers of larvae moving to the right and to the left when no chemical treatment was present on either side of the choice test. Western corn root worm larvae moved slowly through the glass beads, and after 30 minutes, equal numbers of larvae were found in the right and left arms of the Y-tube. The CO<sub>2</sub> concentration in the shell vials containing ambient air was  $0.99 \pm 0.08$  mmol/mol. Larvae also chose equally between the right and left sides of the choice test when carbonated water was present on both sides in shell vial sources ( $P > 0.05$ ). Each shell vial of carbonated water produced  $24.49 \pm 1.31$  mmol/mol of CO<sub>2</sub>.

Please amend the paragraph beginning on page 90, line 25 as follows:

AS5

**CO<sub>2</sub> Analysis of Corn Plants in Soil.** The CO<sub>2</sub> concentration in the soil atmosphere in tubs containing 8-day-old growing corn plants was  $4.36 \pm 0.31$  mmol/mol (measured by GC-MS-SIM). The concentration of CO<sub>2</sub> in tubs containing soil alone was  $1.38 \pm 0.03$  mmol/mol, and the concentration in the ambient air was  $0.94 \pm 0.01$  mmol/mol.

Please amend the paragraph beginning on page 93, line 5 as follows:

AS6

In syringe source bioassays, the larval response to CO<sub>2</sub> increased with each increase in the amount of CO<sub>2</sub> added to the syringe mixtures (1, 3, 10, ....  $\mu$ l of CO<sub>2</sub>) (Graph 18-4) when the control side contained  $1.00 \pm 0.09$  mmol/mol of CO<sub>2</sub>. In the dose--response test, the attractive range of concentrations was from  $1.34 \pm 0.05$  to  $85.6 \pm 1.20$  mmol/mol. The most attractive concentrations of CO<sub>2</sub> were  $2.51 \pm 0.13$  mmol/mol (30  $\mu$ l of CO<sub>2</sub> added to the syringe), and  $4.20 \pm 0.21$  mmol/mol (100  $\mu$ l added to the syringe). This range of attractive concentrations of CO<sub>2</sub> is consistent with the level of CO<sub>2</sub> produced by a germinating corn seed in a shell vial ( $6.04 \pm 0.83$  mmol/mol), cut corn roots in a shell vial ( $2.97 \pm 0.15$  mmol/mol), and also with the concentration found in the headspace above 50 grams (dry wt) of germinating corn seeds ( $5.38 \pm 0.45$  mmol/mol). The concentration of CO<sub>2</sub> measured in soil near the roots of growing corn plants ( $4.36 \pm 0.31$  mmol/mol) was consistent with the optimally attractive range of concentrations



A56

( $2.51 \pm 0.13$  to  $4.20 \pm 0.21$  mmol/mol), indicating that the bioassay technique produced gradients of CO<sub>2</sub> similar to those that are behaviorally active in the soil.

Please amend the paragraph beginning on page 94, line 14 as follows:

In the current study, western corn root worm larvae were not attracted to 300 or 900 mmol/mol of CO<sub>2</sub>, and they exhibited toxic symptoms at these high concentrations. Larvae remained in the cap, or in the top 0.5 cm of glass beads, throughout the bioassay period. They were lethargic when removed from the apparatus, but recovered normal movement after 5--10 minutes. Doane et al. (1975) reported a similar lack of response to high concentrations of CO<sub>2</sub> by plant-parasitic nematodes.

A57

Please amend the paragraph beginning on page 94, line 27 as follows:

The ability to detect and respond to small differences in CO<sub>2</sub> concentration may be important in host location by neonate western corn root worm larvae. Strnad et al. (1986) demonstrated that 1st instars follow a gradient of CO<sub>2</sub> to its source, and that they respond to increases in the gradient by exhibiting a reduction in the number of turns and direction changes. Our results indicate that the larvae not only detect these changes but also when given a choice of 2 different concentrations of CO<sub>2</sub>, are attracted to the higher concentration and follow it toward the source. As shown by Branson (1989) and Strnad and Bergman (1987), neonate western corn root worm larvae die if they do not locate food within 3 days after hatching, and their survival to adulthood is significantly reduced if it takes them more than 24 hours to find the roots of a suitable host plant. In more recent studies (MacDonald and Ellis 1990), western corn root worm larvae survived after 24 hours of starvation, and some were able to survive for as long as 13 days with adequate temperatures and soil moisture. In the soil surrounding a growing corn plant, a CO<sub>2</sub> gradient may form around the entire root mass. Western corn root worm larvae may use their ability to detect differences in concentration to orient directly to the root of the corn plant and avoid losing valuable time searching the entire area in which the roots are growing.

A58

Please amend the paragraph beginning on page 111, line 7 as follows:

**Insects.** Western corn root worms (originally obtained from J. Jackson, USDA-ARS Laboratory, Brookings, South Dakota) (non-diapausing strain) were reared on corn plants grown in soil in an

A59

A59  
concl.

incubator using methods described by Jackson (1985) and modified by Hibbard and Bjostad (1988). Periodic additions were made to the colony with eggs obtained from French Agricultural Research (Lamberton, MN). Eggs from a diapausing strain of western corn root worm were obtained from French Agricultural Research. The eggs (in soil) were kept moist and larvae were used in bioassays within 12 hours of hatching.

Please amend the paragraph beginning on page 111, line 19 as follows:

A60

**Corn.** Untreated, dried corn seeds (*Zea mays*, cv 3055 provided courtesy of Gary D. Lawrance, Pioneer Hi-Bred International, Inc., Johnston, Iowa) were washed with soapy water, soaked for 24 hours in soapy water (1 drop of Ivory Dishwashing Liquid, Procter & Gamble, Cincinnati, OH, per liter of water), and rinsed thoroughly with water. For use in bioassays, the washed seeds were germinated 3 days on germination paper (Steel Blue, Anchor Paper Company, St. Paul, MN) in a closed polyethylene tub (30 by 15 cm), and the plants typically reached a shoot length of 1 cm and a root length of 6 cm.

Please amend the paragraph beginning on page 112, line 1 as follows:

A61

**Bioassay Procedure.** All bioassays were choice tests conducted using a vertical glass "Y" tube apparatus filled with 3-mm glass beads (Bernklau and Bjostad 1998) (Graph 19-1-A). Volatile compounds were prepared in 35-ml polyethylene syringes (cat no. 106-0490, Sherwood Medical, St. Louis, MO) and a syringe pump (Sage Model 355, Fisher Scientific, Pittsburgh, PA) was used to provide, slow (1 ml per min) consistent delivery of the compounds into each choice arm of the bioassay apparatus. Twenty newly-hatched larvae (less than 12-hours-old) were used for each bioassay. Non-diapausing larvae were used for all experiments unless otherwise indicated below. For each choice test a minimum of 10 replicates were conducted.

Please amend the paragraph beginning on page 112, line 24 as follows:

**Corn Headspace Versus CO<sub>2</sub>.** Using the glass bead bioassay (Bernklau and Bjostad 1998)

A62

the headspace over germinating corn seeds was tested in a choice test against a series of CO<sub>2</sub> concentrations to determine if corn volatiles (including CO<sub>2</sub>) were more attractive to the larvae than CO<sub>2</sub> alone. A 35-ml syringe was filled with the headspace over 3-day-old germinating corn

AG

seedlings by means of a 25-cm length of slender Teflon tubing inserted into a hole drilled into the cover of the tub containing the corn seedlings.

Please amend the paragraph beginning on page 113, line 1 as follows:

Three different concentrations of CO<sub>2</sub> were tested on the control side of the choice test. In the 1st test, we used ambient room air on the control side, which contains a lower concentration of CO<sub>2</sub> than the corn headspace (approximately 1.0 mmol/mol). In the 2nd test, we used GC-MS-SIM to match the CO<sub>2</sub> concentration in the syringe on the control side to be equal to that measured in the syringe containing corn headspace. In the 3rd test, the syringe on the control side of the choice test contained a CO<sub>2</sub> concentration twice that measured in the corn headspace. To prepare each of these control concentrations, a 2nd 35-ml polyethylene syringe was partially filled (approximately 5 ml) from a tank containing pure (100%) CO<sub>2</sub> using a glass syringe. Headspace from a plastic tub containing only moist germination paper was drawn into the syringe to fill it, mixing the air and CO<sub>2</sub> thoroughly at the same time. The gas mixtures in the polyethylene syringes were allowed to equilibrate for 15 minutes, and GC-MS-SIM was used to verify the CO<sub>2</sub> concentrations in both syringes prior to each bioassay.

Please amend the paragraph beginning on page 115, line 15 as follows:

**Headspace from Corn in Soil Versus CO<sub>2</sub>.** We considered the possibility that microorganisms and other components of the soil environment may interact with growing corn roots to produce volatile compounds that attract western corn root worm larvae, and that they may not be present in corn that is germinated outside of soil. Using the method described above, the headspace obtained from soil that contained growing corn plants was tested against different concentrations of CO<sub>2</sub> to determine if such volatiles attract western corn root worm larvae. The bottom of a glass dessicator (Cat No. 25031-026, VWR Scientific, Denver, CO) (20 cm high, 25 cm diameter) was filled with water (3 cm deep). A perforated ceramic plate (suspended 6 cm from the bottom) was lined with filter paper (Whatman No. 4, 15 cm diameter, Cat No. 1004-090, Springfield Mill, Maidstone, Kent, England). Two 35-cm pieces of slender Teflon tubing were secured on top of the filter paper with sewing thread tied through the holes in the plate. The filter paper and tubing were covered with 2 cm of a 4:1 soil/peat moss mixture, and the soil was then moistened with 40 ml of water. Untreated, dried corn seeds (50) that had been washed with

ALG  
enc!

soapy water, soaked for 24 hours, and rinsed thoroughly, were evenly spread over the soil and covered to a depth of 1 cm. The cover of the dessicator was replaced. The Teflon tubes were secured with cellophane tape to the sides of the chamber so that they projected out the hole (4 cm diameter) in the cover. When the plants were 8 days old, 35 ml of the soil headspace was drawn into a 35-ml polyethylene syringe through the 35-cm Teflon tubes. A 2nd 35-ml polyethylene syringe was filled (as described above) with 1 of 3 concentrations of CO<sub>2</sub> (ambient CO<sub>2</sub>, CO<sub>2</sub> matching the concentration in the headspace over the damaged corn seeds, or twice the concentration of CO<sub>2</sub> in the soil headspace): The gas mixtures in the polyethylene syringes were allowed to equilibrate for 15 minutes, and GC-MS-SIM was used to verify the CO<sub>2</sub> concentration in both syringes prior to each bioassay.

---

Please amend the paragraph beginning on page 116, line 20 as follows:

---

AL5

Soil Bioassay. A variation of the bioassay apparatus containing soil was used to test larval attraction to corn compounds of limited volatility that might be present in soil in which corn is grown. Washed, soaked corn seeds (9) were planted in a plastic tub (11 cm high, 7 cm diameter) in soil that had been sifted through a 0.32 mm mesh and through a 5 mm mesh screen (W.S. Tyler Inc., Mentor, Ohio 44060). An equal amount of soil was added to a 2nd tub as a control. Both tubs were uncovered after 3 days and the soil was used for bioassays when the corn plants were 8 days old. The corn plants were removed from the soil and the soil was examined under a microscope to remove any pieces of corn roots that might remain. The bottom of a glass test tube (4 cm long, 8 mm diameter, with a 1.5 mm hole in the bottom) was lined with a square (1 by 1 cm) of organza cloth and the tube was filled with the soil. A Teflon connector was snugly fitted over the bottom end of the tube and a NMR cap (with a 1-mm diameter hole) was inserted tightly inside the connector. A 2nd glass test tube was prepared, using soil from the control tub. The 2 glass tubes were inserted snugly inside the glass Y-tube so that the tops were even with the junction of the 'Y', and the rest of the Y-tube was filled to within 1 cm of the top with soil from the corn tub. A 60-ml polyethylene syringe containing a 5 mmol/mol mixture of CO<sub>2</sub> (prepared as described above) was connected to the side of the Y-tube containing corn soil via a 25-cm length of Teflon tubing inserted into the hole in the NMR cap. A 2nd 60-ml polyethylene syringe was filled (as described above) with 1 of 3 concentrations of CO<sub>2</sub> (1, 5 or 10 mmol/mol CO<sub>2</sub>) and connected to the control side of the Y-tube. GC-MS-SIM was used to verify the CO<sub>2</sub>

AS  
6  
con!

concentration in both syringes prior to each bioassay. Bioassays were run for 60 minutes.

Please amend the paragraph beginning on page 117, line 22 as follows:

**Corn Headspace From Western Corn root worm-Damaged Corn Versus CO<sub>2</sub>.** Using the same method described in the 1st experiment, the headspace over germinating corn seeds that had been fed upon by western corn root worm larvae was tested against CO<sub>2</sub> to determine if larval feeding causes corn roots to produce volatile compounds that are more attractive to western corn root worm larvae than those from undamaged roots. Corn seeds were germinated in covered plastic tubs as described above. After 3 days, 80 2nd-instar western corn root worm larvae were transferred onto the roots of the germinating corn seeds, the container was closed and the larvae were allowed to feed for 24 hours. A 35-ml polyethylene syringe was filled with the headspace containing the corn volatiles from the damaged corn, and a 2nd 35-ml polyethylene syringe was filled with 1 of 3 concentrations of CO<sub>2</sub> (ambient CO<sub>2</sub>, CO<sub>2</sub> matching the concentration in the headspace over the damaged corn seeds, or twice the concentration of CO<sub>2</sub> in the corn headspace). The gas mixtures in the polyethylene syringes were allowed to equilibrate for 15 minutes, and GC-MS-SIM was used to verify the CO<sub>2</sub> concentration in both syringes prior to each bioassay.

Please amend the paragraph beginning on page 118, line 11 as follows:

**Corn Surface Extracts.** Surface extracts of germinating corn seeds were tested for larval attraction. Germinating corn seeds (3-day-old, 50 grams dry wt as determined at the end of the experiment) were firmly packed into a glass tube (30 cm long, 30 mm diameter, tapering to 12 mm diameter) and diethyl ether (glass-distilled) was dribbled through the seedlings until 8 ml of extract had been collected. The extract was concentrated to 2 ml by evaporation with a gentle stream of nitrogen. Different aliquots of the extract (0.003, 0.03, 0.1, 0.3, 3.0, and 30 gram equivalents corn) were applied to a strip of filter paper (Whatman no. 5, 0.5 by 2 cm) and an equal volume of control solvent, concentrated similarly, was applied to another strip of filter paper. After the solvent had evaporated, the strips were placed in the glass connection tube on the end of either branch of the Y-tube and the NMR cap was replaced. The bioassay was conducted as described above with equal concentrations of CO<sub>2</sub> (3 mmol/mol) in the syringes on both sides.

AL  
4

Please amend the paragraph beginning on page 118, line 31 as follows:

AC8  
Cryogenic Collections of Corn Volatiles. Germinating corn seeds (3-day-old, 50 grams dry wt as determined at the end of the experiment) were packed into a glass tube (30 cm by 30 mml, tapering to 12 mm). A strip of filter paper (0.5 by 2 cm) and a boiling chip were placed in the bottom of a glass sample tube (12 mm by 35 cm, closed at the bottom) and the sample tube was attached to the bottom of the seed-holding tube with a Teflon connector. For a control, a strip of filter paper and a boiling chip were placed in an empty sample tube. Both sample tubes were immersed in a liquid nitrogen bath (3.5 liters). As the air in the treatment tube condensed, a vacuum was created, which pulled air through the corn seedlings and down into the sample tube. When 2 ml of liquid air had collected in the treatment and control tubes, they were removed from the nitrogen bath, the treatment tube was disconnected from the corn seedling tube, and both tubes were placed into precooled (in liquid nitrogen) styrofoam blocks until the condensed air had boiled away. The filter paper strips were removed from the tubes and immediately inserted into the glass connection tubes on either side of the bioassay apparatus. Bioassays were conducted using the shell vial method (described above) with equivalent concentrations of CO<sub>2</sub> on both sides of the choice test.

Please amend the paragraph beginning on page 119, line 23 as follows:

AB9  
Petri Dish Bioassay. The attraction of western corn root worm larvae to volatile compounds other than CO<sub>2</sub> was previously reported by our laboratory on the basis of experiments conducted using a petri dish bioassay apparatus (Hibbard and Bjostad 1988, 1989; Bjostad and Hibbard 1992). The results we have now obtained using the Y-tube apparatus conflict with these reports, and we conducted experiments using the petri dish bioassay apparatus to re-investigate the results reported previously (Hibbard and Bjostad 1988). Three plastic petri dishes (5 cm diameter) were connected with 2-cm lengths of Teflon tubing (10 mm diameter) inserted into holes in their sides (Graph 19-5A). Holes were cut with a brass tube attached to a soldering iron. The bottoms of the 2 end dishes had 12 mm holes melted through their centers. The apparatus was supported on a ring stand. Cryogenic collections of corn seedlings were prepared as described above, except that no filter paper strip was placed in the bottom of the collection tube. When the tube had warmed to room temperature, it was flushed for 10 sec with 100% CO<sub>2</sub> from a tank at 4 psi, then inverted for 30 sec. For the control side, an empty sample

A789  
Cm-1.

tube was similarly flushed with CO<sub>2</sub> for 10 sec and inverted for 30 sec. Immediately after inversion for 30 sec, each tube was capped and allowed to sit for 15 min to allow the CO<sub>2</sub> to equilibrate. The petri dish apparatus was assembled and a bubble level was used to insure that the apparatus was not tilted to 1 side or the other. When GC-MS-SIM measurements indicated that the CO<sub>2</sub> concentrations in the tubes were equal (measured through pinholes in the caps from within 5 cm of the top of the tubes) both tubes were connected with a Teflon connector to the holes in the bottom of the end dishes of the bioassay apparatus. The covers were placed on all 3 dishes and the apparatus was allowed to sit for 5 min to allow volatile compounds to begin diffusing. After 5 min, 10 2nd-instar western corn root worm larvae were placed in the center of the middle Petri dish and the cover was replaced. The number of larvae in each of the chambers and in the sample tubes was recorded every 5 min for a total of 30 min. All bioassays were conducted in dim lighting. CO<sub>2</sub> concentrations within the 3-petri-dish apparatus were measured by removing samples through a pinhole in each Teflon connector. A 5- $\mu$ l sample was taken from each side every 60 sec throughout the 30-minute period and analyzed using GC-MS-SIM. Twenty replicates of the behavioral bioassay were conducted, and CO<sub>2</sub> measurements were taken for 8 replicates.

Please amend the paragraph beginning on page 121, line 5 as follows:

A710

Statistical Analysis. Analysis of variance was conducted for each experiment using orthogonal comparisons (Winer, 1971). In most of the experiments, corn volatiles were present on one side of the bioassay apparatus, and on the other side there was a defined CO<sub>2</sub> concentration that was equal to, greater than, or less than that on the corn volatile side. For each orthogonal comparison, a treatment was compared with its corresponding mean ( $P = 0.05$ ), for both the CO<sub>2</sub> data and the behavioral data. There were thus 3 orthogonal comparisons for the CO<sub>2</sub> data and also for the behavioral data from each of these experiments, with an experiment-wise error rate of  $P = 0.05$ . The petri dish bioassay was analyzed similarly, except that 7 orthogonal comparisons were made, comprising the 7 bioassay intervals, for both the CO<sub>2</sub> data and the behavioral data. Means and standard errors are expressed as mean + standard error in the text that follows.

Please amend the paragraph beginning on page 121, line 26 as follows:

**Corn Headspace Versus CO<sub>2</sub>.** For the non-diapausing strain of western corn root worm, significantly more larvae ( $P < 0.05$ ) chose the corn headspace side (Graph 19-1B) when the control syringe contained ambient room air. There was no significant difference between the number of larvae that chose the corn headspace and larvae that chose the control when the CO<sub>2</sub> concentrations were the same (Graph 19-1C). Larvae chose the control side significantly more often when the control contained twice the concentration of CO<sub>2</sub> as the corn headspace.

Please amend the paragraph beginning on page 122, line 4 as follows:

**Corn Headspace Versus CO<sub>2</sub> with Diapausing Larvae.** Similar results were obtained with the diapausing strain of western corn root worm. Significantly more of the larvae ( $P < 0.05$ ) chose the corn headspace side when the control syringe contained ambient room air (Graph 19-1D). There was no significant difference between the number of larvae that chose the corn headspace and larvae that chose the control when the CO<sub>2</sub> concentrations were the same (Graph 19-1E). Larvae chose the control side significantly more often when the control contained twice the concentration of CO<sub>2</sub> as the corn headspace.

Please amend the paragraph beginning on page 122, line 15 as follows:

**Corn Headspace-Coated Glass Beads Versus CO<sub>2</sub>.** Significantly more larvae ( $P < 0.05$ ) chose the corn-coated beads and corn headspace side of the bioassay when the control side contained ambient room air (Graph 19-2A). There was no significant difference between the number of larvae that chose the corn headspace and larvae that chose the control when the CO<sub>2</sub> concentrations were the same (Graph 19-2B). Larvae chose the control side significantly more often when the control contained twice the concentration of CO<sub>2</sub> as the corn headspace.

Please amend the paragraph beginning on page 122, line 25 as follows:

**Headspace from Corn in Soil Versus CO<sub>2</sub>.** The larvae chose the corn-coated beads and corn headspace significantly more often ( $P < 0.05$ ) when the control syringe contained ambient room air (Graph 19-3A). Significantly more larvae chose the CO<sub>2</sub> control over the corn headspace when the CO<sub>2</sub> concentrations were the same (Graph 19-3B). Larvae chose the control side significantly more often when the control contained twice the concentration of CO<sub>2</sub> as the corn



headspace.

Please amend the paragraph beginning on page 123, line 1 as follows:

**Soil Bioassay.** The larvae chose the soil from growing corn roots significantly more often ( $P < 0.05$ ) (Graph 19-4A) when the syringe on the corn side contained a higher concentration of  $\text{CO}_2$  than the control side (Graph 19-4B). There was no significant difference between the number of larvae that chose the corn headspace and larvae that chose the control when the  $\text{CO}_2$  concentrations were the same. Larvae chose the control side more often when the control contained twice the concentration of  $\text{CO}_2$  as the treatment side.

Please amend the paragraph beginning on page 123, line 11 as follows:

**Corn Headspace From Western Corn root worm-Damaged Corn Versus  $\text{CO}_2$ .** The larvae chose the headspace from damaged corn seedlings significantly more often ( $P < 0.05$ ) when the control syringe contained ambient room air (Graph 19-5A). Significantly more larvae chose the  $\text{CO}_2$  control over the corn headspace when the  $\text{CO}_2$  concentrations were the same (Graph 19-5B). Larvae chose the control side significantly more often when the control contained twice the concentration of  $\text{CO}_2$  as the corn headspace.

Please amend the paragraph beginning on page 124, line 3 as follows:

**Petri Dish Bioassay.** There was no significant difference between the number of larvae that chose the cryogenic collection of corn volatiles and larvae that chose the control ( $P > 0.05$ ) in the petri dish bioassay (Graph 19-6B). During the 30 min that the bioassay was run, there was no significant difference between the  $\text{CO}_2$  concentration on the corn side and the control side inside the petri dish apparatus (Graph 19-6C).

IN THE CLAIMS:

✓ Please cancel Claims 1-19

Please add the following new Claims

20. A method to attract termites, comprising:  
providing an enclosure having openings for termites to pass therethrough;

providing, in said enclosure, an emitting source for emitting at least one gas of: (i) CO<sub>2</sub>, and (ii) one or more mimics thereof including haloalkanes and alkylcarbonates;

5        wherein when said enclosure, with said emitting source therein, is positioned at a location such that for the at least one gas emitted by said emitting source, a concentration of said at least one gas is emitted from said openings so that when said concentration is encountered by the termites, the termites are attracted to said emitting source;

10        wherein said concentration is approximately at least 0.2% by volume of an ambient atmosphere, and said concentration is maintained in an area about said enclosure for at least two weeks so that the termites are attracted to said emitting source rather than to a structure sought to be protected from the termites.

21.    The method of Claim 1, wherein said concentration is in a range extending to about 50%.

22.    The method of Claim 1, wherein said concentration is in a range extending to about 5%.

23.    The method of Claim 1, wherein said concentration is in a range extending to about 2%.

24.    The method of Claim 1, wherein said concentration is in a range from about 0.5% to 1%.

25.    The method of Claim 1, wherein said emitting source includes one of: carbonate, calcium carbonate and a bicarbonate formulation.

26.    The method of Claim 1, further including a step of providing in said enclosure soil.

27.    The method of Claim 7, further including providing said soil with a moisture content of approximately 20%.

A78  
contd  
Rule 1.126  
Sub  
B1

28. The method of Claim 1, further including a step of providing in said enclosure one of: an insecticide, insect growth regulator, a feeding stimulant, another termite attractant, and a material that changes termite movement.

29. The method of Claim 9, further including a step of including in said enclosure one of: hexaflurone, hydramethylnon, and phermones.

30. The method of Claim 1, wherein said enclosure includes one of: bacterial, fungal, algal, and other microorganism formulations for generating said concentration.

31. The method of Claim 1, wherein said enclosure is positioned within two meters of a termite colony.

32. The method of Claim 1, wherein said step of providing said emitting source includes providing one of: spent brewer's grain, ground germinated corn seeds, sodium bicarbonate, and spent grain extract.

33. The method of Claim 1, wherein said emitting source includes a material that is one of: charred and burned.

34. The method of Claim 14, wherein said material includes one of: wood, a cellulosic matrix, a polymeric matrix, wood, paper, cardboard, a fabric, a textile, wool, silk, bone, hair, horn, and claws.

35. A termite trap, comprising:

an enclosure having openings for termites to pass therethrough;

an emitting source for emitting at least one gas of: (i) CO<sub>2</sub>, and (ii) one or more mimics thereof including haloalkanes and alkylcarbonates;

5 wherein when said enclosure, with said emitting source therein, is positioned at a location such that for the at least one gas emitted by said emitting source, a concentration

of said at least one gas is emitted from said openings so that when said concentration is encountered by the termites, the termites are attracted to said emitting source;

10 wherein said concentration is at least about 0.2% by volume of air encountered by termites, and said concentration is maintained in an area about said enclosure for at least two weeks so that the termites are attracted to said emitting source rather than to a structure sought to be protected from the termites.

36. The termite trap of Claim 16, wherein said concentration is in a range extending to about 50%.

37. The termite trap of Claim 16, wherein said concentration is in a range extending to about 5%.

38. The termite trap of Claim 16, wherein said concentration is in a range extending to about 2%.

39. The termite trap of Claim 16, wherein said concentration is in a range from about 0.5% to 1%.

40. The termite trap of Claim 16, wherein said emitting source includes one of: carbonate, calcium carbonate and a bicarbonate formulation.

41. The termite trap of Claim 16, said enclosure includes soil.

42. The termite trap of Claim 22, where said soil has a moisture content of approximately 20%.

43. The termite trap of Claim 16, wherein said enclosure includes one of: an insecticide, insect growth regulator, a feeding stimulant, another termite attractant, and a material that changes termite movement.

44. The termite trap of Claim 24, wherein said enclosure includes one of: hexaflurone, hydramethylnon, and phermones.
45. The termite trap of Claim 16, wherein said enclosure includes one of: bacterial, fungal, algal, and other microorganism formulations for generating said concentration.
46. The termite trap of Claim 16, wherein said enclosure is positioned within two meters of a termite colony.
47. The termite trap of Claim 16, wherein said emitting source includes one of: spent brewer's grain, ground germinated corn seeds, sodium bicarbonate, and spent grain extract.
48. The termite trap of Claim 16, wherein said emitting source includes a material that is one of: charred and burned.
49. The termite trap of Claim 29, wherein said material includes one of: wood, a cellulosic matrix, a polymeric matrix, wood, paper, cardboard, a fabric, a textile, wool, silk, bone, hair, horn, and claws.
50. The termite trap of Claim 16, wherein no more than about 10% of the surface area of said enclosure comprises said openings.
51. The termite trap of Claim 16, wherein at least some of said openings are approximately 3 mm in diameter.
52. The termite trap of Claim 16, wherein said concentration attracts one of: *Reticulitermes tibialis*, *Reticulitermes flavipes*, and *Reticulitermes virginicus*.
53. The termite trap of Claim 16, wherein the termites are attracted through said openings.

A78  
contd

Rule 1.126

54. The termite trap of Claim 16, wherein said enclosure includes a sufficient amount of said emitting source for emitting the at least one gas over a period of at least two months.

55. A termite trap, comprising:

an enclosure having openings for termites to pass therethrough;

means for emitting at least one gas of: (i) CO<sub>2</sub>, and (ii) one or more mimics thereof including haloalkanes and alkylcarbonates;

5 wherein when said enclosure, with said means for emitting therein, is positioned at a location such that for the at least one gas emitted by said means for emitting, a concentration of said at least one gas is emitted from said openings so that when said concentration is encountered by the termites, the termites are attracted to said emitting source;

10 wherein said concentration is at least about 0.2% by volume of air encountered by termites, and said concentration is maintained in an area about said enclosure for at least two weeks so that the termites are attracted to said emitting source rather than to a structure sought to be protected from the termites.

56. A method to distracting termites, comprising:

providing, in an enclosure having an emitting source for emitting at least one gas of: (i) CO<sub>2</sub>, and (ii) one or more mimics thereof including haloalkanes and alkylcarbonates;

5 providing in said enclosure openings for said at least one gas to pass therethrough; wherein when said enclosure, with said emitting source therein, is positioned at a location such that for the at least one gas emitted by said emitting source, a concentration of said at least one gas is emitted from said openings so that when said concentration is encountered by the termites, the termites are distracted by said emitting source from a  
10 food source;

wherein said concentration is approximately at least 0.2% by volume of an ambient atmosphere, and said concentration is maintained in an area about said enclosure for at least two weeks.